Send Orders of Reprints at reprints@benthamscience.net

Current Biotechnology, 2013, *2,* 192-200

Simulation of Microgravity for Studies in Gravitational Biology: Principles, Devices and Applications

Ralf Anken*

Institute of Aerospace Medicine, German Aerospace Center DLR, Linder Hoehe, D-51147 Cologne, Germany

Abstract: Scientists and technicians have been innovative to develop experimental platforms in order to achieve functional weightless conditions in their Earth-bound laboratories. As a result, various experimental platforms are available in order to perform studies with molecules or single cells up to humans and to study gravity-related mechanisms.

These ground-based simulators of microgravity are not only tools to prepare spaceflight experiments, but they have been established as stand-alone facilities for gravitational research.

This review provides an overview of some of the most frequently used microgravity simulators, most of which are in use at DLR´s Institute of Aerospace Medicine at Cologne, Germany. Their individual capacities but also experimental limitations, especially regarding their range of applicability for biological specimens, are exemplarily reviewed here. Overall, it is necessary to compare data achieved by using simulators with the data obtained in real microgravity. Furthermore, it should be carefully considered which kind of simulation might be the optimum for a given model organism or cell.

Keywords: Space biology, gravitational biology, gravity, microgravity, clinostat, random positioning machine.

INTRODUCTION

Since the dawn of life on Earth some 4 billions of years ago, the earthly gravity vector has been the only constant and always present environmental factor (radiation, temperature, light, humidity etc. are factors which change during a day/night cycle or over longer timespans), which thus has influenced the phylogenetic development of all existent organisms. Gravity acts both as a factor of physical restriction and as a ubiquitous cue for orientation and postural control. Investigating the effects of a decreased or increased gravitational environment on biosystems thus leads to cues and insights into its paramount role regarding physiology, shape and function of living beings.

In the field of gravitational biology, research under the conditions of altered gravity such as microgravity (space missions) and hypergravity (centrifuges) has yielded numerous findings concerning the impact of gravity on biological processes, gravity-sensing mechanisms and on the gravity-based orientation of organisms. Yet, however, we have only approximately understood the underlying basics since spaceflight experiments are costly and, in consequence, respective flight opportunities are scarce and it is usually not possible to carry out series of experiments. In order to somehow bridge the gap, devices have been developed in an effort to subject biosystems to "weightlessness" on ground.

The classic clinostat has been introduced in the late 19th century by Julius Sachs. Since then, a variety of ground-based facilities have been designed to simulate "weightlessness", which either randomize the direction of Earth´s gravity in a way that the exposed system does not perceive accelerations (clinostat principle) or compensate the gravity force by a counteracting force (magnetic levitation). Drop-towers are the only means to provide true free-fall-conditions on ground, but this only lasts a few seconds depending on the height and whether the sample is shot up, which is by far not sufficient to investigate, e.g., developmental processes.

Since simulators cannot provide true "weightlessness", results gained always have to be interpreted with great caution in order to discriminate effects of "weightlessness" from effects generated by the simulation technique itself (e.g., centrifugal accelerations, vibrations, shearing forces etc.). It is especially important to compare results gained using a simulator with those from experiments under real "weightlessness" in order to assess the quality of a simulation and always to bear in mind that not every simulator is suited to simulate "weightlessness" from the perspective of a given biological model system.

Here, we give an overview of the most frequently used microgravity simulators, with a focus on those which are readily available at DLR´s Institute of Aerospace Medicine at Cologne, Germany. Their individual capacities and limitations will be addressed, based on a series of Europe-wide investigations in the course of which the range of applicability of the various ground-based microgravity simulators for different biological specimens has been evaluated in a comprehensive ESA project [1]. First, in order to avoid misunderstandings and inconsistencies, we will briefly discuss nomenclatorical issues.

*Address correspondence to this author at the Institute of Aerospace Medicine, German Aerospace Center, Linder Hoehe, D-51147 Cologne, Germany; Tel: +49 (0)2203/601-3420; Fax: +49 (0)2203-696212;
E-mail: ralf.anken@dlr.de

2211-5501/13 $58.00+.00

SIMULATED MICROGRAVITY

A variety of terms has been used to describe the gravitational situation of an object in a simulator, such as "vector-averaged gravity" [2,3], "nullification of the gravity stimulus" [4], "modeled microgravity" [5,6], "near-Earth free-fall orbit" [6], "microweight simulator" [7], "randomized microgravity" [8] or "low shear environment" [9,10]. Other authors just use a more neutral terminology without judging the achieved acceleration condition, which exactly describes the experimental methods, such as "clinorotation" or "wall vessel rotation" (e.g., [11-13]). In physical terms, "…microgravity is the condition in which the absolute sum of all mass-dependent accelerations does not exceed a certain small "noise", i.e., meaning around 10^{-6} times *g* [14]. Weightlessness also has been described as "No mechanical support of mass…" [15] and as a "…result from a net sum of all forces present equaling zero, not from absence of gravity…" [16]. In "microgravity", however, not all forces need to be equal to zero. There are still, e.g., capillary forces, hydrostatic pressure, cell surface binding forces etc. [17].

A simulator cannot diminish the Earth gravity vector, but it can change its influence on a biological system [15]. A simulator thus cannot generate true microgravity, but it may provide "functional weightlessness" from the perspective of the object, when the physical environment results in an impact (according to [15]) below the sensitivity of relevant biological process.

In order to avoid confusion and to provide a basis to be generally accepted, it was proposed [1] to use the term "simulated microgravity" to describe the conditions applied when (correctly) using a simulator. Texts and/or figure legends should exactly explain the simulator (Ground Based Facility, GBF) used, including the main parameters to enable an experimenter to reproduce the simulation (size and shape of the sample container, mode of operation, rotational speeds and tube diameters for Random Positioning Machines (RPMs)/clinostat etc.).

In analogy, it was proposed [1] to use the term "microgravity" regarding such experiments that have been performed in real microgravity such as aboard the International Space Station ISS, on satellites, on sounding rockets, in drop towers or during the parabolic flight of aircrafts. The term "microgravity" is used irrespective of the concrete *g*-level, that means it might be different from 10^{-6} times *g*. Thereby a logical wording opposite to hypergravity is achieved.

MICROGRAVITY SIMULATORS

A variety of experimental platforms have been developed to achieve simulated microgravity conditions. They are used to demonstrate the gravisensitivity of a biosystem, to develop and test flight hardware and countermeasures and to support statistically the results obtained in (real) microgravity.

In principal, organisms of all evolutionary levels as well as cells can be maintained in these facilities. Nevertheless, experiences show that one should carefully consider and choose the sort of device with respect to its suitability for a given object. Evaluation parameters are the size of a sample and the reaction time of the object, furthermore cultivation and environmental requirements and conditions for maintenance of the specimens.

Here, we review the following simulation techniques, since they are commonly used in laboratories dealing with gravitational biology:

1. Clinostats with one or two axes (2-D, 3-D clinostats)
2. Random Positioning Machine (RPM)
3. Rotating Wall Vessel (RWV)

CLINOSTATS

All kinds of clinostats have in common that a sample is constantly rotated perpendicularly to the gravitational field in order to hinder a biological system in perceiving the gravitational acceleration vector (Fig. **1**). The different configurations differ with respect to the number of rotation axes, the speed and the direction of rotation [15,16,18,19]. Clinostats with one rotation axis are usually called 2-D clinostats. A second rotation axis which is oriented perpendicular to the first one, operating constantly at a given speed and direction, characterizes a 3-D clinostat. Furthermore, if the speed and the direction are randomly changed during the run, the device is called Random Positioning Machine.

Early clinostats rotated slowly with 1–10 rpm (rotations per minute) and were mainly used to study the gravitropism of plants. Though slow rotation prevents the gravity-triggered growth response, an omnilateral mechanical stress in some sensitive plant tissues instead of a *g*-stimulus-free environment by this approach appears likely [20].

The concept of the fast rotating clinostat to be used on small objects such as cells [15], small animals and plants was introduced by Wolfgang Briegleb in the 1950s. By the fast and constant rotation, sedimentation of a sample (and on the next level of organs/organelles within the sample) is prevented. By video observation of particles located in the vicinity of the center of a small tube or chamber during clinorotation, it has been shown that they are forced to move in circular paths. The diameter of these circles decreases with increasing rotation speed, finally reaching a stage where gravity-induced movements can be neglected. A cell (mass) thus rotates around itself/its center, being surrounded by a liquid boundary layer [16]. It is assumed that a biosystem is no longer able to perceive gravity under these conditions. In order to meet scientific requirements, various kinds of clinostats besides the ordinary clinostats [4,7,21] have been developed, enabling, e.g., microscopic observation (Clinostat Microscope, Fig. **2A**), online kinetic measurements (Photomultiplier Clinostat [22]), fixation during rotation (Pipette Clinostat, Fig. **2B**), developmental studies under submersed conditions (Submersed Clinostat, Fig. **2C** [12, 21]), clinorotation of adherent cells (Fig. **2D**) and even portable clinostats [1].

ROTATING WALL VESSEL

Rotating Wall Vessels (RWVs) or Rotating Bioreactors (Rotating Cell Culture System, RCCS, initially developed by

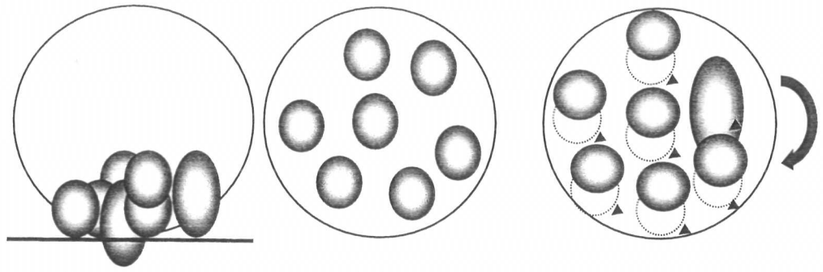

Fig. (1). Scheme explaining the principle of a fast rotating 2-D clinostat. Under Earth gravity, particles sediment. In real microgravity, particles homogeneously distribute due to the lack of sedimentation. At an appropriate speed of rotation, no movement of the particles relative to the Earth gravity vector occurs, the particles just move around themselves; thus the particles are under simulated microgravity.

NASA) have been designed for cell cultures [23] and for aquatic organisms such as fish eggs/embryos [13,24]. The specimens are kept in suspension, while they continuously fall. The submersed version of the RWV shown here (Fig. **2E**) was designed and constructed at the German Aerospace Center [12] to study fish development under simulated microgravity.

RANDOM POSITIONING MACHINE

It was hypothesized that the quality of simulating microgravity for larger objects might be increased by rotating a sample around two axes. Consequently, corresponding devices have been developed in Japan and in the Netherlands (for review see [25]), characterized by two independently rotating frames (Fig. **2F**). In case both frames rotate at a constant speed and direction [26,27], the term 3-D clinostat is being used; in case that both frames are operated with randomized speeds and directions the term Random Positioning Machine (RPM) is appropriate. As has been suggested [1], a description of the exact mode of operation of the dedicated simulation device should be supplied in a publication.

VALIDATION OF THE CHOSEN SIMULATION APPROACH

A final judgement of the quality of simulation only is possible if the response of the objects in real microgravity is known.

In the course of numerous studies, various organisms and cells have so far been investigated using different microgravity simulators. Most recently, several investigations were carried out in the framework of an ESA GBF project [1], in the course of which both the abilities and limitations of GBFs, in relation to biological model systems and based on a comparison with results obtained under (real) microgravity during spaceflight, were critically assessed. The major outcome of this study is summarized in Table **1**.

In the project mentioned [1], bacteria have not been studied and they are thus not being covered here. There is only extremely few known on the responses of bacteria to altered gravity without allowing any general conclusions.

According to literature [e.g., 30] bacterial responses to microgravity are to be increasingly studied since humans orbit, and it may become even more interesting when commercial spaceflight becomes available.

Gravitational environments have been shown to correlate with distinctive changes in bacterial gene expression, as well as proliferative changes in their growth rate. The latter is primarily due to the reduction of extracellular mass transfer, as well as the motility of the bacterium. Recent studies [30] have also shown an increase in expression of virulence factors in particular species and increased biofilm production in others. The common devices used for simulating microgravity upon bacteria are the clinostat, the high-aspect ratio vessel bioreactor (HARV; a kind of rotating wall vessel, RWV) and the rotating wall vessel bioreactor (another kind of RWV). Since rotation can cause centrifugation of the bacteria toward the vessel wall if the speed is too great or cause their sedimentation downward if the speed is too low, much research has been done to find the optimal rate that would produce motionlessness (see [1]). It was noted by microbiologists [30] in line with the review presented here that one should bear in mind that simulations cannot fully reproduce the effects of actual spaceflight.

Besides the GBF-project in focus here [1], a number of investigations have been performed using an RPM on mammalian cells in suspension. It has been reported [e.g., 31] that human mesenchymal stem cells cultured in a 3-D clinostat (i.e., an RPM) showed marked proliferation compared with cells cultured under normal conditions. It was concluded [31] that using an RPM may provide an environment to successfully expand stem cell populations *in vitro* without culture supplements that can adversely affect stem cell-derived transplantations. According to [31] the method has significant potential for regenerative medicine and developmental biology. It remains, however, to be questioned and investigated, to which extent an RPM actually provides microgravity from the perspective of the cell.

Although the gravitropism of plants has been extensively studied under conditions of altered gravity, plant gravitropism is not comprehensively addressed here in Table **1** as gravitropism usually has been studied using slow rotating clinostats. Slow clinorotation prevents gravitropism due to the slow response time of plants. However, further analysis revealed distinct morphological differences depending on the speed of rotation. As a conclusion slow rotating clinostats should be critically assessed with respect to simulation of microgravity or omnilateral gravistimulation.

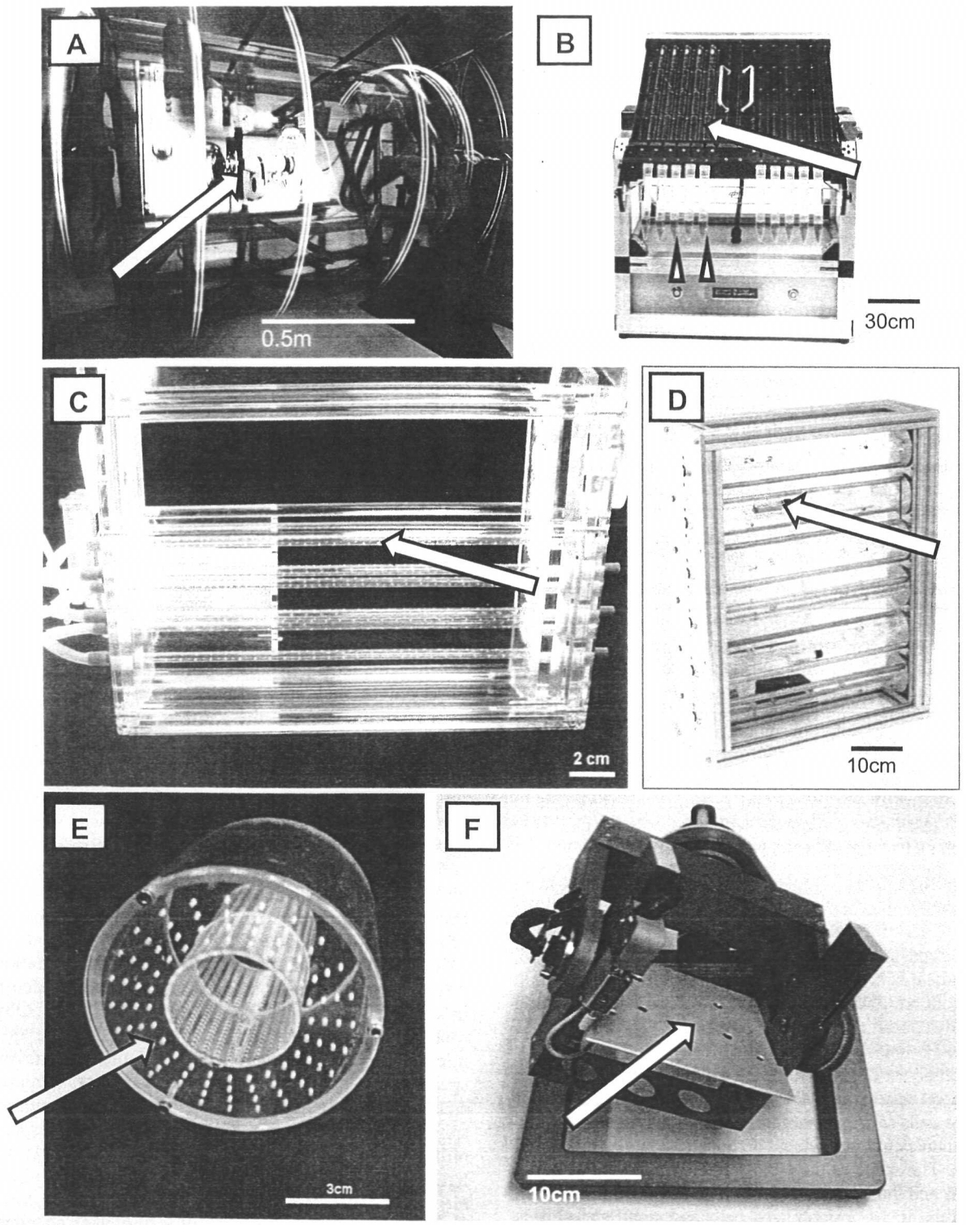

Fig. (2). Images of rotating/positioning devices used in gravitational biology to simulate microgravity. **A**. 2-D Clinostat Microscope [15] rotating around and thus having a sample (cell) under investigation directly during rotation/simulated microgravity [e.g., 15]. This device is located at the DLR Institute of Aerospace Medicine, **B**. Pipette 2-D Clinostat [e.g., 28] showing a row of rotating pipettes across the top to hold suspended cells. The cells can be fixed during rotation and poured into tubes (arrowheads), **C**. Submersed clinostat to be placed inside an aquarium with rotating tubes for keeping aquatic organisms (for details see [12]), **D**. Clinostat for adherent cells (i.e., cells that adhere to a given substrate) [29]. Here are shown chambers, fixed to horizontal rotors, to accommodate adherent cells, **E**. A rotating wall vessel for submersion in an aquarium [12], and **F**. A random positioning machine RPM for cells in suspension and small organisms (commercially available from Dutch Space, Leiden, The Netherlands). **A**. – **E**. were designed and constructed at DLR, Cologne. The RPM in **F**. was purchased from Dutch Space and is hosted at DLR, Cologne, as well as in other laboratories. Arrows: Center where simulated microgravity is being achieved or where samples are being installed.

Table 1. Biological Responses in Microgravity Simulators (GBFs) in Comparison to Real Microgravity. From [1], Modified

Object	Parameter	2-D Clinostat	RPM
Unicellular organisms:	Gravitaxis		
Paramecium		++	-
Euglena		++	-
Unicellular alga: *Chara*	Statolith displacement	++	+
Plant: *Arabidopsis*	Cell proliferation/growth	n.a.	++
	Gene expression	n.a.	+
Insect: *Drosophila*	Behavior	n.a.	+
	Gene expression	n.a.	++
Fish: *Oreochromis*	Behavior	n.a.	n.a.
	Development	+	n.a.
Mammalian:	Gene and protein expression, mRNA transcription levels, cell morphology		
Adherent cells		+	+
Cells in suspension		+	n.a. (but see text)
Bacteria	not evaluated in the course of the GBF study [1], see text for information on this subject		

(++), (+), (-): Response to simulation identical, similar or different from responses observed under conditions of real microgravity. n.a.: not applicable or data from experiments under real microgravity not available.
Details on the studies carried out in this GBF project can be found in [1]. Here, we provide the main conclusions from this project.
The table is not exhaustive, but will give some hints to the researcher who wants to carry out a study using a simulation technique.

Unicellular Free-Swimming Cells (*Paramecium* and *Euglena*)

The fast-rotating 2-D clinostat can be regarded as a suitable simulator of microgravity for unicellular gravitactic *Euglena* and *Paramecium* with a size of 50 µm to 200 µm, whereas the RPM (in the random mode of operation) is less suited. The change of swimming pattern in real microgravity is similar to the one on a fast rotating clinostat, while the cells are passively drifting on the RPM due to the strong fluid shifts.

In more detail, two-dimensional clinorotation (60 rpm; clinostat microscope, see above) revealed no change in the swimming paths of *Paramecium* compared to the swimming pattern observed in real microgravity. Exposure on the RPM, however, indicated an increase in directional turns. The random speed and random direction mode on the RPM induced strong drifting and course corrections. *Euglena* cells, three times smaller than *Paramecium,* even drifted away passively, thereby making an analysis of the swimming velocities and the degree of orientation impossible. Two-dimensional clinorotation of immobilized cells (*Paramecium*) and small glass beads resulted in a continuous circling, which demonstrated one-directional acceleration. The speed of the clinostat determined the diameter of the circles and thus the relative movements of the particles. In contrast, on the RPM, immobilized cells and small glass beads were shown to bounce and move out of the rotation center [Sascha Hoppe, DLR, personal communication to Ralf Anken, see also [1]].

These results demonstrate that samples on the RPM experience positive and negative acceleration forces induced by continuously changing the direction and speed of rotation.

Rhizoids of Characean Green Algae

Also concerning the statolith-based gravity sensing system of the unicellular *Chara* rhizoids, the 2-D fast-rotating clinostat is a very good simulator for microgravity as the displacement of statoliths is similar to the one observed in real microgravity on sounding rocket flights. The 3-D clinostat and the RPM showed results in the same direction; however, there is no advantage of a second rotation axis, which is much more complicated to implement.

In more detail [consult [1]] clinorotated rhizoids exhibited a statolith displacement which was very similar to the one that was earlier observed under real microgravity conditions. Although clinorotation generally resulted in a clear basipetal displacement of statoliths, the complex of rhizoid statoliths rotated on the classical clinostats (slow rotating) often appeared more dispersed than on fast clinostats or in real microgravity. After clinorotation, more dispersed statoliths sedimented more slowly, which resulted in a delayed initiation of the gravitropic response. Obviously, rotation around two axes did not improve the quality of microgravity simulation but reduced the volume in which specimens experience a good-quality microgravity simulation from a cylindrically shaped volume to a spherically shaped volume of the same diameter of several millimeters depending on the rotational speed [1; also Lars Krause, DLR, personal communication to Ralf Anken]. As is the case concerning all simulators addressed in the present study, physical constraints result in the clear statement: The higher the rotational speed, the higher the residual g-force, *ergo* the smaller the useful sample volume.

Arabidopsis thaliana

The results obtained on *Arabidopsis* using the RPM are fully homologous to those gained by experiments under (real) microgravity with respect to the parameters of cell growth and proliferation, which represent the last steps in a gravity signal transduction chain. The impact on the perception level remains to be studied.

According to [1], data obtained from cell cultures revealed alterations in cell growth and proliferation that could be compared to those found in seedlings, indicating that a significant part of the response to altered gravity does not depend on specialized cells containing mechanoreceptors. In cell cultures exposed on the RPM alteration of the relative proportion of cell cycle phases (which probably leads to a change in the duration of the cycle), the change in expression of numerous genes acting as regulators in cell cycle checkpoints of phase transitions, and the deregulation of ribosome biogenesis by means of the alteration of nucleolar structure and of transcriptional and post-translational changes of key proteins of this process, such as nucleolin and fibrillarin have been described.

So far, the results with respect to *Arabidopsis* on the RPM are totally homologous to those obtained in real microgravity. Specifically for cell cultures, it is mandatory to expose experimental and control samples to the same environment, including temperature, shaking, and magnetic and inertial forces, parameters often difficult to control in GBFs.

Drosophila

For *Drosophila* gene expression studies, the RPM is a good simulator as has been shown in the course of the GBF project cited [1]. An RPM also acts as a good simulator concerning behavioral issues as behavior of *Drosophila* is similar in the RPM to the one observed under real microgravity, but particular entities of behavior may be affected by the RPM´s random inertial movements, which remains to be studied in detail. Corresponding experiments on a 2-D clinostat have not yet been performed.

Fish

A RWV cannot be used in investigations on fish swimming behavior, because freely swimming late-staged larvae perform countermovements during rotation. The same applies for the RPM at low rotation speeds (i.e., some 20 rpm and below). High speeds (e.g., 60 rpm) have a negative influence on behavior due to the random inertial movements, i.e., they induce the escape response. Fish behavior also cannot be investigated using a 2-D clinostat, since the tubes are too narrow to allow a fish to swim around (and larger sized tubes would generate hypergravity).

The speed of a commercially available RPM (Dutch Space) is too low to make a fish "feel" weightless. Developmental studies on the inner ear therefore make no sense. A RWV can be a good simulator of microgravity when early-staged larvae are used, which cannot yet swim around freely due to the large yolk-sac. However, it depends on the species used. In the RWV, the development of inner ear otoliths in egg-laying Zebrafish is similar to that which has to be expected under real microgravity conditions (i.e., they grow faster as under 1 times *g*), but in mouthbreeding cichlids, the RWV has no effect on otolith development. Keeping cichlids in a 2-D clinostat yields similar effects on otolith development as have been observed under real microgravity.

Mammalian Cell Cultures

Regarding adherent cells of mammalian origin (i.e., cells adherent to carriers or adherent on the bottom of slideflasks [for details see 29]), the 2-D clinostat as well as the RPM [32] are well suited devices to simulate microgravity. However, special adaptations are necessary, g-gradients and thus maximal g-values have to be calculated and considered [29].

The 2-D clinostat has been found to simulate microgravity well in case of cell cultures in suspension. Comparable studies on the RPM are currently under investigation. 3-D cell culturing might be achieved in an RPM and may provide interesting aspects in the field of tissue engineering.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The investigations hitherto undertaken in order to assess the quality of a simulation of microgravity have shown that it depends on a variety of parameters such as kind and size of a sample, cell or tissue, the time a sample needs to respond to the gravitational stimulus as well as the threshold of sensitivity to gravity of a signaling process in question.

The fast rotating 2-D clinostat is a good simulator of microgravity and thus can be recommended for most (small) organisms or cells under study. Some larger biosystems such as *Arabidopsis* also receive a sufficient simulation of microgravity using an RPM.

Both the fast-rotating 2-D clinostat and the RPM change the direction of the Earth gravity vector from the perspective of the sample. If the process sensing gravity of a given biosystem is fast and sensitive, one must not neglect that a rotation may lead to a general mechanical stimulation, which may result in stress responses or even the death of the cell [25].

The rotation speed has to be adjusted correspondingly to the speed of sensing by a biosystem/signaling process under investigation which is not known in most cases. Regarding fast dynamic processes, this is a problem.

The higher the rotation speed, the higher is the quality of simulated microgravity right in the center of a tube based on qualitative considerations [15], but this goes along with a reduction of the space around the horizontal axis, where simulated microgravity can be obtained (further away from the center, centrifugal forces play an increasingly prominent role and the quality of microgravity is decreased, consult Table **2**). In consequence, fast rotating clinostats can only be used on small specimens/cell culture volumes. Using a larger liquid volume in an RPM, shear forces by the movement of the liquid may occur [29] and the impact of these effects has to be studied. It has been suggested [1,27,34,35] that the RPM is especially suited for studies on *Arabidopsis*. It also may play a role in tissue engineering.

Such considerations reveal that a device developed to simulate microgravity does not simply do so. An experimenter has to carefully evaluate the capacities and the limitations of these simulators concerning the biosystem to be analyzed. Therefore, it has been stated [1] that it is advantageous to use several GBFs and a variety of modes of operations, before one draws a final conclusion, which is ideally done not earlier than after having carried out an experiment in real microgravity.

In order to allow comparisons and a critical evaluation and interpretation of results, it is also necessary to disclose in

a manuscript all relevant information on the simulator and the mode of operation used in detail. Always, one also has to critically review possible side effects of physical parameters affecting the biosystem [15,16].

Table 2. Residual Gravity (X*g) at the Periphery in a Rotating, Horizontal Tube (2-D Clinostat with One Axis), Depending on the Radius of the Tube (r) and the Rotation Speed (Rotations Per Minute, rpm), Calculated According to $a = \varpi^2 \cdot r$, where a is the Residual Acceleration Level, ϖ is the Angular Speed and r is the Radius

r [cm]	rpm	X*g
0.1	60	4.0243 E(-3)
0.1	90	9.0547 E(-3)
0.2	60	8.9486 E(-3)
0.2	90	1.8109 E(-2)
0.3	60	1.2073 E(-2)
0.3	90	2.7164 E(-2)
0.4	60	1.6097 E(-2)
0.4	90	3.6219 E(-2)
0.5	60	2.0121 E(-2)
0.5	90	4.5273 E(-2)
0.6	60	2.4146 E(-2)
0.6	90	5.4328 E(-2)
0.7	60	2.8170 E(-2)
0.7	90	6.3383 E(-2)
0.8	60	3.2194 E(-2)
0.8	90	7.2437 E(-2)
0.9	60	3.6219 E(-2)
0.9	90	8.1492 E(-2)
1.0	60	4.0243 E(-2)
1.0	90	9.0547 E(-2)

OUTLOOK

Ground-based facilities aiming at the achievement of functional weightlessness provide a wide range of experimental advantages and possibilities. Thus, they are currently equipped with state-of-the-art analytical devices for online observations and analysis, such as fluorescence, Raman spectroscopy and high speed video-observation during simulation of microgravity or during real microgravity. Using an ordinary video camera providing some 25 frames per second, particular fish seemingly rested during microgravity in the course of a drop tower flight; viewing these animals with a high speed camera, it was seen that they in fact turned around their longitudinal axis quickly [Reinhard Hilbig, University of Stuttgart-Hohenheim, Germany; personal communication to Ralf Anken].

A European project aims at developing a space-worthy fluorescence microscope. It is planned to fly a version of this microscope on a TEXUS sounding rocket flight in 2014 in order to online investigate cytoskeleton dynamics of a variety of human cells [Wolfgang Hanke, University of Stuttgart-Hohenheim, Germany; personal communication to Ralf Anken]. Such devices also need to be accommodated in ground based devices for simulation of microgravity.

It also would be advantageous to couple experimental setups to be subjected to simulated microgravity with life support systems to allow, e.g., (long-term) multigeneration experiments. A new concept to transform biowaste such as foliage and urea into nutrients for plants is being under development at DLR [36]. It would be interesting to investigate the performance of such a setup under altered gravity; in a first step, the system could be installed on a short arm human centrifuge, which is readily available at DLR, Cologne [37]. This centrifuge also can hold large experimental devices for, e.g., cell biological studies under hypergravity [37].

Overall, it is the main goal of science centers which can provide means to alter gravity and, especially, which have experience in simulating microgravity, to continuously trigger a technical evolution of devices to simulate microgravity for the benefit of scientists working in the field of gravitational biology.

FURTHER ASPECTS

We have dealt here with means to simulate microgravity for biological model systems, being it a cell, a plant or an animal (fly, fish). Certainly, there are also facilities available to generate effects of microgravity on so-called "higher" organisms such as rodents or even humans. Rodents or humans are by far too large to be placed in one of the devices discussed above (and larger devices would not generate simulated microgravity for physical reasons).

Effects of simulated microgravity regarding cardiovascular issues as well as bone and muscle physiology may well be tackled in using hindlimb suspension/hindlimb unloading (HU) (rodents and other small mammals) or bed rest (humans).

The rodent hindlimb unloading model (see, for review on technical aspects [38]) is a well-established ground-based model for investigating disuse (including disuse due to unloading under microgravity) effects on bone and muscle. Hindlimb unloading results in significant reductions in disuse-sensitive cancellous bone mass, architecture, and material properties owing to early increases in bone resorption followed by prolonged depressions in bone-formation rate [39]. According to [39] skeletal muscle atrophy and reduced functional properties (i.e., strength) have been demonstrated as early as 4 days after hindlimb unloading begins and may remain depressed for some time upon reambulation. To date, no method has successfully prevented deficits in both muscle and bone during long-duration unloading [39].

According to the review of [39] resistance exercise that includes eccentric contractions provides an anabolic stimulus for both skeletal muscle and bone in both humans and rodent models and generally has proven more effective in promoting increased bone and muscle mass than endurance exercise protocols such as running. Eccentric contractions (during muscle lengthening) generate larger muscle forces than do concentric contractions (muscle shortening), providing greater increases in bone mineral density. Until the launching of improved training equipment late in 2008, ISS astronauts were unable to train at the high intensities (>70% maximum) demonstrated as osteogenic in ground-based studies [39].

Overall, HU seems to be a proper means to mimic conditions of weightlessness on rodents and related small mammalians regarding cardiovascular, bone and muscle issues. HU as a technique to investigate possible effects of microgravity on vestibular issues is being disputed. HU itself may place the gravity-sensing utricular statolith masses of the vestibular system into a position, where it is not fully able to perceive the Earth gravity vector. Such a positioning may, however, affect proprioceptors (e.g., in the neck of the animal) resulting in sensations not comparable to those received under real microgravity. It is known that the vestibular system contributes to blood pressure regulation. Prior studies reported that lesions that eliminate inputs from the inner ears attenuate the vasoconstriction that ordinarily occurs in the hindlimbs of conscious cats during head-up rotations [40]. Further studies on this topic are needed.

The success of a Space mission depends on maintaining the crew healthy, especially during longterm trips. Current exercise countermeasures (i.e., cycle ergometer, treadmill, and resistance exercise device) used aboard the International Space Station ISS are not fully effective in avoiding losses of bone mineral density or geometry, resulting in a significant increase in estimated fracture risk that remains even one year after returning to Earth (for review see [39]).

These changes in humans exposed to microgravity parallel alterations in bone and muscle mass observed during prolonged bed rest. Bed rest and countermeasure exercises differentially affect the various functions of skeletal muscle. Moreover, the time course during recovery needs to be considered more thoroughly in future studies. More research is also needed to develop countermeasures that maintain muscle strength as well as other muscle functions including power [41].

At the Institute of Aerospace Medicine at Cologne, Germany, the new facility :envihab has been designed to answer complex questions regarding human physiology under altered environmental conditions.

:envihab is a medical research facility where diverse environmental conditions on humans can be analyzed and explored as well as possible countermeasures are being developed. The concept of :envihab is also to deal with complex problems of a life support system and the interaction between humans and the environment from a medical, biological and psychological point of view. Major focus will be put on research topics that deal with providing for health and performance of humans. The modular house-in-house concept makes it possible to use the different units and the technical equipment without leaving the building. 12 test persons can permanently be exposed to equal and controlled environmental conditions.

ABBREVIATIONS

DLR	=	German Aerospace Center
ESA	=	European Space Agency
GBF	=	Ground Based Facility
HARV	=	High-aspect ratio vessel bioreactor (a kind of RWV)
HU	=	Hindlimb Unloading (a synonym of hindlimb suspension)
ISS	=	International Space Station
NASA	=	National Aeronautics and Space Administration
RCCS	=	Rotating Cell Culture System (a synonym of RWV)
RPM (upper case)	=	Random Positioning Machine
rpm (lower case)	=	rotations per minute
RWV	=	Rotating Wall Vessel
TEXUS	=	Technologische Experimente unter Schwerelosigkeit (a sounding rocket program)
2-D, 3-D	=	Two, three dimensional

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Herranz R, Anken R, Boonstra J *et al.* Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. Astrobiology 2013; 13: 1-17.

[2] Nakamura H, Kumei Y, Morita S, Shimokawa H, Ohya K, Shinomiya K. Suppression of osteoblastic phenotypes and modulation of pro- and anti-apoptotic features in normal human osteoblastic cells under a vector-averaged gravity condition. J Med Dent Sci 2003; 50: 167-76.

[3] Sarkar D, Nagaya T, Koga K, Nomura Y, Gruener R, Seo H. Culture in vector-averaged gravity under clinostat rotation results in apoptosis of osteoblastic ROS 17/2.8 cells. J Bone Miner Res 2000; 15: 489-98.

[4] Dedolph R, Oemick D, Wilson B, Smith G. Causal basis of gravity stimulus nullification by clinostat rotation. Plant Physiol 1967; 42: 1373-83.

[5] Plett P, Abonour R, Frankovitz S, Orschell C. Impact of modeled microgravity on migration, differentiation, and cell cycle control of primitive human hematopoietic progenitor cells. Exp Hematol 2004; 32: 773-81.

[6] Zayzafoon M, Gathings W, McDonald J. Modeled microgravity inhibits osteogenic differentiation of human mesenchymal stem cells and increases adipogenesis. Endocrinology 2004; 145: 2421-32.

[7] van Loon J, Veldhuijzen J, Kiss J *et al.* Microgravity Research starts on the ground. Apparatuses for long-term ground based hypo- and hypergravity studies. Proceedings of the 2nd European Symposium on the Utilisation of the International Space Station; 1998 Nov 16-18; ESTEC, Noordwijk, The Netherlands. ESA SP-433 1999; pp. 415-20.

[8] England L, Gorzelak M, Trevors J. Growth and membrane polarization in *Pseudomonas aeruginosa* UG2 grown in randomized microgravity in a high aspect ratio vessel. Biochim Biophys Acta 2003; 1624: 76-80.

[9] Hammond T, Hammond J. Optimized suspension culture: the rotating-wall vessel. Am J Physiol Renal Physiol 2001; 281: F12-25.

[10] Nickerson C, Ott C, Wilson J, Ramamurthy R, Pierson D. Microbial responses to microgravity and other low-shear environments. Microbiol Mol Biol Rev 2004; 68: 345-61.

[11] Anken R, Baur U, Hilbig R. Clinorotation increases the growth of utricular otoliths of developing cichlid fish. Microgravity Sci Technol 2010; 22: 151-4.

[12] Brungs S, Hauslage J, Hemmersbach R, Hilbig R, Anken R. Effects of simulated weightlessness on fish otolith growth: Clinostat versus rotating-wall vessel. Adv Space Res 2011; 48: 792-8.

[13] Li X, Anken R, Wang G, Hilbig R, Liu Y. Effects of wall vessel rotation on the growth of larval zebrafish inner ear otoliths. Microgravity Sci Technol 2011; 23: 13-8.

[14] Albrecht-Buehler G. The simulation of microgravity conditions on the ground. ASGSB Bulletin 1992; 5: 3-10.

[15] Briegleb W. Some qualitative and quantitative aspects of the fast-rotating clinostat as a research tool. ASGSB Bulletin 1992; 5: 23-30.

[16] Klaus D. Clinostats and bioreactors. Gravit Space Biol Bull 2001; 14: 55-64.

[17] Albrecht-Buehler G. Possible mechanisms of indirect gravity sensing by cells. ASGSB Bulletin 1991; 4: 25-34.

[18] Häder D-P, Rosum A, Schafer J, Hemmersbach R. Gravitaxis in the flagellate *Euglena gracilis* is controlled by an active gravireceptor. J Plant Physiol 1995; 146: 474-80.

[19] Klaus D, Schatz A, Neubert J, Höfer M, Todd P. *Escherichia coli* growth kinetics: a definition of "functional weigthlessness" and a comparison of clinostat and space flight results. Naturwissenschaften 1997; 143: 449-55.

[20] Hensel W, Sievers A. Effects of prolonged omnilateral gravistimulation on the ultrastructure of statocytes and on the graviresponse of roots. Planta 1980; 150: 338-46.

[21] Hemmersbach R, von der Wiesche M, Seibt D. Ground-basedexperimental platforms in gravitational biology and human physiology. Signal Transduction 2006; 6: 381-7.

[22] Horn A, Ullrich O, Huber K, Hemmersbach R. PMT Photomultiplier Clinostat. Microgravity Sci Technol 2011; 23: 67-71.

[23] Schwarz R, Goodwin T, Wolf D. Cell culture for three-dimensional modeling in rotating-wall vessels: an application of simulated microgravity. J Tissue Cult Methods 1992; 14: 51-7.

[24] Moorman S, Burress C, Cordova R, Slater J. Stimulus dependence of the development of the zebrafish (*Danio rerio*) vestibular system. J Neurobiol 1999; 38: 247-58.

[25] van Loon J. Some history and use of the Random Positioning Machine, RPM, in gravity related research. Adv Space Res 2007; 39: 1161-5.

[26] Borst A, van Loon J. Technology and Developments for the Random Positioning Machine, RPM. Microgravity Sci Technol 2009; 21: 287-92.

[27] Hoson T, Kamisaka S, Masuda Y, Yamashita M, Buchen B. Evaluation of the three-dimensional clinostat as a simulator of weightlessness. Planta 1997; 203: 187-97.

[28] Paulsen K, Thiel C, Timm J *et al.* Microgravity-induced alterations in signal transduction in cells of the immune system. Acta Astronautica 2010; 67: 1116-25.

[29] Eiermann P, Kopp S, Hauslage J, Hemmersbach R, Gerzer R, Ivanova K. Adaptation of a 2-D clinostat for simulated microgravity experiments with adherent cells, Microgravity Sci Technol 2013, published online 02 June 2013, DOI 10.1007/s12217-013-9341-1.

[30] Benoit M, Klaus D. Microgravity, Bacteria, and the influence of motility. Adv Space Res 2007; 39: 1225-32.

[31] Yuge L, Kajiume T, Tahara T *et al.* Microgravity potentiates stem cell proliferation while sustaining the capability of differentiation. Stem Cells and Development 2006; 15: 921-29.

[32] Grosse J, Wehland M, Pietsch J *et al.* Gravity-sensitive signaling drives 3-dimensional formation of multicellular thyroid cancer spheroids. The FASEB J 2012; 12: 5124-40.

[33] Leguy C, Delfos R, Pourquie M, *et al.* Fluid Motion for Microgravity Simulations in a Random Positioning Machine. Gravit Space Biol 2011; 25: 36-9.

[34] Hoson T, Kamisaka S, Masuda Y, Yamashita M. Changes in plant growth processes under microgravity conditions simulated by a three-dimensional clinostat. Bot Mag 1992: 105; 53-70.

[35] Kraft T, van Loon J, Kiss J. Plastid position in *Arabidopsis* columella cells is similar in microgravity and on a random-positioning machine. Planta 2000; 211: 415-22.

[36] Hauslage J, Lebert M, Waßer K, Bornemann G, Hemmersbach R, Anken R. A novel approach in bioregenerative life support for terrestrial applications and for experiments aboard a compact satellite. Proc Plant Biol Congr Freiburg 2012: 237.

[37] Zander V, Anken R, Pesquet T, Brungs S, Latsch J. Short radius centrifuges – A new approach for life science experiments under hyper-G conditions for applications in space and beyond. Recent Patents on Space Technol 2013; 3: DOI 2210-6871/13.

[38] Morey-Holton E, Globus R. Hindlimb unloading rodent model: technical aspects. J Appl Physiol 2002; 92: 1367-77.

[39] Swift J, Nilsson M, Hogan H, Sumner L, Bloomfield S. Simulated resistance training during hindlimb unloading abolishes disuse bone loss and maintains muscle strength. J Bone Mineral Res 2010; 25: 564-74.

[40] Yavorcik K, Reighard D, Misra S *et al.* Effects of postural changes and removal of vestibular inputs on blood flow to and from the hindlimb of conscious felines. Am J Physiol Regul Integr Comp Physiol 2009; 297: R1777–R1784.

[41] Buehring B, Belavý D, Michaelis I, Gast U, Felsenberg D, Rittweger J. Changes in lower extremity muscle function after 56 days of bed rest. J Appl Physiol 2011; 111: 87-94.

Received: April 5, 2013 Revised: May 29, 2013 Accepted: July 12, 2013